Biological activity of essential oil from aerial parts of *Artemisia aucheri* Boiss. from Iran

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Summary

Artemisia aucheri Boiss. is a shrub from Asteraceae family has been widespread in Iran. In traditional medicine, *A. aucheri* is used for its astringent, disinfectant, antimicrobial and antiparasitic properties. The aim of this study was to evaluate the chemical composition and antimicrobial activity of essential oil from aerial parts of *A. aucheri* against different microorganisms including Gram positive, Gram negative bacteria, filamentous fungi and yeast by disc diffusion and micro broth dilution assays. The antioxidant activity of *A. aucheri* essential oil was evaluated by DPPH free radical scavenging system. Fifty five components were identified by GC and GC/MS analysis and quantified from the essential oil of *A. aucheri,* representing 98% of total oil. The major components were geranyl acetate (17.2%), E-citral (17.1%), linalool (12.7%), geraniol (10.7%), Z-citral (10.5%). The antimicrobial results showed that *Pseudomonas aeruginosa* was resistant to the oil and *Staphylococcus aureus* and *Candida albicans* showed the best sensitivity to the oil. The *A. aucheri* has powerful antioxidant activity than that of Trolox. Some investigations were be done for evaluating the efficacy of essential oil.

Key words: *Artemisia aucheri, chemical composition, antimicrobial, antioxidant activity*

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INTRODUCTION

One of the most important shrub plants is *Artemisia* genus that has different species. The genus of *Artemisia* has 34 annual and perennial species that has been distributed in tropical and subtropical regions of Iran [1]. *Artemisia aucheri* Boiss. is an aromatic plant from Asteraceae family, an indigenous plant that distributed in Iran. In traditional medicine, the plant was used as astringent, disinfectant, antiseptic, antiparasitic, antipoisoning. There are some investigations on chemical composition of essential oil from aerial parts of *A. aucheri*. 1,8-cineole (22.8%), chrysanthenone (18.16%), α-pinene (8.33%) and mesithylene (7.4%) were the major components of *A. aucheri* essential oil obtained by microextraction method [2]. Verbenole (21.5%), camphor (21%), 1,8-cineole (8.3%) and trans verbeneole (8.1%) were identified in essential oil from plants of Semnan, Iran [3]. Camphor (45.5%) and 1,8-cineole (14.3%) were the main components of essential oil from Mohammad poor et al. study [4]. Linalool (44.1%), geranyl acetate (10.7%), E-citral (9.7%) and Z- citral (7.7%) as the major components of *A. aucheri* essential oil from Khorasan Province was reported [5]. Some pharmacological studies exhibited that methanol extract of *A. aucheri* had irreversible metabolic hurt on promastigotes of *Leishmania major* [6]. The *A. aucheri* extract decreased TG, TC, LDL cholesterol levels and increased HDL- cholesterol level in rabbits and this study showed that this effect is related to antioxidant and antiinflammatory effect of *A. aucheri* extract [7]. The antimicrobial activity of *A. aucheri* essential oil against phytopatogenic fungi exhibited that this oil inhibited the growth of *Rhizoctonia solani* at concentration of 41.4 μl/ml [5]. In this investigation, we evaluated the chemical composition, antimicrobial and antioxidant activities of essential oil from aerial part of *A. aucheri*.

MATERIALS AND METHODS

Plant material

The aerial parts of *Artemisia aucheri* Boiss in seeding stage were collected from suburb of Kashan, Iran. The voucher specimen were prepared and authenticated. The herbarium sample was deposited at the Herbarium of Agriculture Department, Research and Development of Barij Essence, Kashan, Iran.

Extraction, isolation and identification of the oil

Aerial parts of plant were subjected to hydrodistillation by clevanger type apparatus (6h). The oil were dried over anhydrus sodium sulfate.

The oil analysis was carried out using GC-FID and GC/MS. The GC apparatus was Agilent technology (HP) 6890 system, capillary column of HP-5MS (60 m × 0.25 mm, film thickness 0.25 μm). The oven temperature program was initiated at 40°C, held for 1 min, then raised up to 230°C at a rate of 3°C/min held for 10 min. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. The detector and injector temperatures were 250 and 230°C, respectively. The GC/MS analysis was conducted on a HP 6890 GC system coupled with 5973 network mass selective detector with a capillary column the same as above, carrier gas helium with flow rate 1 ml/min with a split ratio equal to 1/50, injector and oven temperature programmed was identical to GC. The compounds of the oil were identified by comparison of their retention indices (RI), mass spectra fragmentation with those on the stored Wiley 7n.1 mass computer library and NIST (National Institute of Standards and Technology).

Microbial strains and growth media

Staphylococcus aureus ATCC 25923, *Staphylococcus epidermidis* ATCC 14490, *Streptococcus pneumoniae* ATCC 49615, *Streptococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 8739, *Shigella flexeneri* NCTC 8516, *Pseudomonas aerugeninosa* ATCC 9027, *Aspergillus niger* ATCC 16404, field isolate of *Aspergillus flavus* and *Candida albicans* ATCC 10231 were used as tested microorganisms. Bacterial suspensions were made in Brain Heart Infusion (BHI) broth to a concentration of approximately 10^8 cfu/ml using standard routine spectrophometrical methods. Suspensions of fungi were prepared in Sabouraud dextrose broth. Subsequent dilutions were prepared from the above suspensions, which were then used in the tests.

Disc diffusion method

The disc diffusion method was employed for the determination of antimicrobial activities of the oil in question. Briefly, using a sterile cotton swab, 0.1 ml from 10^8 cfu/ml microbial suspensions was spread on the Mueller Hinton Agar (MHA) plates for non fastidious bacteria, Todd Hewitt Agar was used for fastidious bacteria and Sabouraud dextrose Agar for fungi. Sterile filter paper discs (6 mm in diameter) were impregnated with 2.5, 5, 7.5, 15 μl of the oil and placed on the inoculated plates. These plates, after remaining at 4°C for 2 h, were incubated for 24 h at 37°C (for bacteria) and for 48 h at 30°C (for fungi). The diameters of the inhibition zones were measured in millimeters. All tests were performed in triplicate.

Determination of minimum inhibitory (MIC) and lethal (MLC) concentrations

The minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) values of oil were determined by micro broth dilution assay. The oil was twofold serially diluted with 10% DMSO which contains 16–0.0125 μl/ml of oil. These dilutions were prepared in a 96-well microtitre plate. MOPS (morpholine propane sulphunic acid)-buffered RPMI 1640 with L-glutamine but lacking bicarbonate (for fungi) [8], cation adjusted Muller Hinton broth (non fastidious bacteria) [9] and Todd Hewitt broth (fastidious bacteria) [10] were used as broth media. After shaking, 100 μl of oil was added to each well. The above mentioned microbial suspension was diluted (1×10^6 CFU/ml for bacteria; 1×10^4 for fungi) and then 100 μl was added to each well and incubated at 35 °C. MICs were defined as the lowest concentration of compound that inhibits bacteria after 24 and fungi after 48 h. MLC values were the first well that showing no growth on solid media.

Radical scavenging capacity of the oils by DPPH assay

Radical scavenging activity was determined by a spectrophotometric method based on the reduction of the methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) [11]. Trolox in the quantity of 1 mM (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a stable antioxidant were used as positive controls and purchased from Sigma. Briefly, fifty microlitres of 1:5 concentrations of the oil in methanol were added to 5 ml of a 0.004% methanol solution of DPPH. After a zero, 30, 70 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical by DPPH in percent (I%) was calculated in following way:

$$I\% = [A_{blank} - A_{sample}/A_{blank}] \times 100,$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Tests were carried out in triplicate.

RESULTS AND DISCUSSION

Fifty-four components were identified in the essential oil of *A. aucheri*, representing 98% of total oil. The major components were geranyl acetate (17.2%), E-citral (17.1%), linalool (12.7%), geraniol (10.7%) and Z-citral (10.5%). 1,8-cineole (2.1%), borneole (2.5%), camphor (3.2%) are other major components of oil (tab. 1).

Table 1.

Chemical composition of *Artemisia aucheri* Boiss. essential oil

compound	RI	(%)
1-octene	771	0.04
santolina triene	896	0.11
α-pinene	925	0.66
camphene	942	0.65
β-phellandrene	966	0.2
β-pinene	971	0.2
6-methyl-5-hepten-2-one	976	1.0
β-myrcene	981	1.1
yomogi alcohol	987	0.06
α-phellandrene	997	0.04
herboxid second isomer	999	0.15
α-terpinene	1010	0.13
cymene	1016	1.6
limonene	1021	0.23
1,8-cineole	1025	2.1
γ-terpinene	1051	0.45
trans linalool oxide	1066	0.35
α-terpinolene	1085	0.63
linalool	1091	12.7
β-thujone	1100	1.5
α-thujone	1110	0.4
cis-1-methyl-4(1-methyl)-2-cyclohexan-1-ol	1115	0.2
1-terpineol	1133	0.3
camphore	1139	3.2
lavandulol	1162	1.4
borneol	1165	2.5
4-terpineol	1172	1.4
p-cymen-8-ol	1179	0.36
β-fenchyl alcohol	1185	0.74
tarragon	1188	0.23
β-citronellol	1222	0.86
z-citral	1232	10.5
geraniol	1248	10.7
e-citral	1265	17.1
lavandulyl acetate	1272	0.4
borneol acetate	1276	1.4
cis-2,6-dimethyl-2,6-octadiene	1330	0.4
3,7-dimethyl-2,6-octadien-1-ol	1343	0.4
geranyl acetate	1368	17.2
methyl eugenol	1387	0.2
cis-jasmone	1389	0.35
trans-caryophyllene	1417	0.43
geranyl propionate	1455	0.22
α-curcumene	1469	0.45
zingiberene	1481	0.13
β-selinene	1483	0.24
cis-davanone	1571	0.3
spathulenol	1575	0.22
caryophyllene oxide	1581	0.33
valencene	1620	0.2
methyl jasmonate	1632	0.15
5-(t-butyl)-4-methoxy-1,2-dihydroxybenzene	1661	0.23
α-bisabolol	1669	0.2
β-bisabolene	1675	0.5

In disc diffusion method, the antimicrobial activity of oil increased in a dose dependent manner. The antimicrobial activity of *A. aucheri* oil exhibited that this oil has antibacterial activity against *S. aureus*, *S. saprophyticus*, *S. pneumoniae*, *Sh. flexeneri*, *A. flavus* and *C. albicans* and the inhibition zone diameter of some concentrations of essential oil is larger than compared antibiotics. The inhibition zone of 2.5 μl of essential oil on filamentous fungi (*A. niger* and *A. flavus*) and *C. albicans* is larger than the amphotricin B. In bacteria sensitive to essential oil, the inhibition zones of 5 or 7.5 μl and 15 μl of the essential oil are larger than the antibiotics. In Gram-positive bacteria, *S. faecalis* is more resistant to essential oil than the others. The inhibition zone of Gram-negative bacteria especially *E. coli* and *P. aeruginosa* is smaller than gentamycin. The essential oil had no effect on *P. aeruginosa* and none of the concentrations of essential oil created the inhibition zones against *P. aeruginosa*. In micro broth dilution assay, the MIC, MLC values of *C. albicans* and *S. aureus* were 0.5, 0.5 μl/ml and is lower than the other microorganisms. The *A. aucheri* oil had cidal effect on both of them. *E. coli*, *P. aeruginosa* had the high MIC, MLC values but *P. aeruginosa* was more resistant than *E. coli*. The oil had an inhibitory effect on *A. niger* because the MLC value is eight fold of MIC. In Gram-positive bacteria, *S. faecalis* had high MIC and MLC than the other. Therefore, it is resistant to the oil but in contrast with the Gram negative is sensitive to the oil (tab. 2). The DPPH radical scavenging activities of *A. aucheri* oil and trolox are shown in figure 1. *A. aucheri* oil reduces the concentration of free radical with an efficacy higher than that of trolox. The *A. aucheri* oil exhibited maximum DPPH radical scavenging activity, i.e. 41.2%, whereas Trolox exhibited 10.5% inhibition at 70 min. The *A. aucheri* oil has higher antioxidant activity than Trolox. Our results showed that this oil has a powerful antioxidant.

Table 2.

Antimicrobial activity of *A. aucheri* Boiss essential oil against different types of microorganism

	inhibition zone [mm]					minimal concentrations [μl/ml]	
	***A. aucheri* essential oil [μl]**				**Antibiotic**	***A. aucheri* essential oil**	
	2.5	5	7.5	15		MIC	MLC
S. aureus	14.5±0.7	23.8±0.4	30.0±0.0	37.5±0.7	26.8±1.9^{V}	0.5	0.5
S. saprophyticus	11.5±2.1	23.0±1.4	29.5±0.7	32.0±0.0	17.8±0.4^{V}	0.5	1
S. pneumonia	13.0±0.0	30.5±0.7	38.3±0.4	41.5±0.7	18.5±0.5^{V}	1	2
S. faecalis	7.0±0.0	9.0±0.0	13.5±0.7	17.5±0.7	15.0±2.0^{V}	2	2
E. coli	NE	8.0±0.0	8.5±0.7	10.5±0.7	21.4±0.5^{G}	16	16
Sh. flexeneri	14.0±0.0	18.0±0.0	20.3±0.4	23.5±0.7	17.8±1.0^{G}	2	4
P. aeruginosa	NE	NE	NE	NE	24.3±2.6^{G}	>32	>32
A. niger	9.5±0.7	18.5±0.7	36.0±0.0	41.0±1.4	7.8±1.5^{A}	0.5	4
A. flavus	7.0±0.0	11.0±0.0	16.3±0.4	30.5±0.7	7.5±1.7^{A}	1	2
C. albicans	22.5±0.7	31.5±2.1	38.0±0.0	45.0±1.4	13.8±1.0^{A}	0.5	0.5

V – vancomycin 30 μg/disc, G – gentamycin 10 μg/disc, A – amphotricin B 100 U/disc, MIC – Minimal Inhibitory Concentration, MLC – Minimal Lethal Concentration, NE – no effect

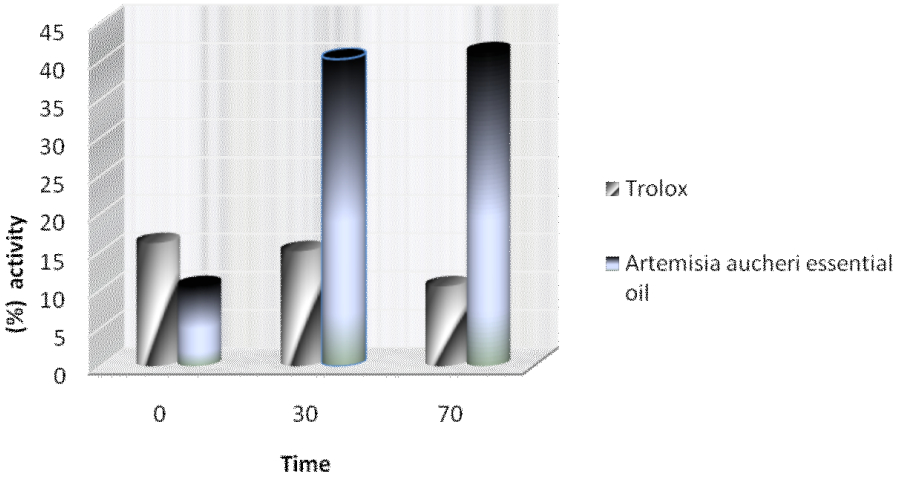

Figure 1. Antioxidant activity of *A. aucheri* Bioss. essential oil by DPPH free radical scavenging

CONCLUSION

Chemical composition analysis of *A. aucheri* essential oil showed that there are some differences in chemical composition of oil described in other reports, but the major components of this oil is like to reported essential oil from Khorasan Province [5] and is different from the one from Semnan Province [3]. Verbenole (21.5%), camphor (21%) and 1,8-cineole (8.3%) as the major components of essential oil from Semnan province and in compared with essential oil from this study exhibited that the amount of 1,8-cineole (2.1%) and camphor (3.2%) are less that Sefidkon et al study and verbenole was not detected in our oil.

The antimicrobial activity of *A. aucheri* essential oil is related to major or minor components of the oil or synergism between the components. Our survey on other investigations showed that geraniol had antibacterial [12] and antifungal activity [13] but geranyl acetate is an organic compound from monoterpene group of weak antifungal activity [14]. Linalool exhibited antimites [15], antibacterial [13], antifungal [14] and anti-flea activity [16]. α-bisabolol had antibacterial and antifungal activity [17]. The antimicrobial activity of oil is related to major components, synergism, or antagonist of components. Perhaps, the presence of linalool or geraniol in oil increased the antimicrobial activity of oil. Lipophilic compounds trend to cellular membrane but due to low solubility of these compounds in aquatic environments, the antimicrobial activities decreased. The antimicrobial activity of *A. aucheri* oil against Gram-positive bacteria is more than Gram-negative ones.

A. aucheri oil had a good antioxidant activity. Therefore, it can be used as an antioxidant, antimicrobial agent in food or drug products.

Today, the resistant isolates of microorganism have increased [18] and chemical agents that are used for treatment of such infectious diseases had some adverse effects. They are some reasons that have encouraged researchers to find new natural compounds. Considering the effectiveness of *A. aucheri* essential oil against different microorganisms, especially against fungi and Gram-positive bacteria, some researches are needed to evaluate the effectiveness of essential oil as a source of active biological compounds in animal models.

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DZIAŁANIE OLEJKU ETERYCZNEGO Z NADZIEMNYCH CZĘŚCI *ARTEMISIA AUCHERI* BOISS. Z IRANU

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S t r e s z c z e n i e

Artemisia aucheri Boiss. jest krzewem z rodziny Asteraceae rozpowszechnionym w Iranie. W medycynie tradycyjnej *A. ancheri* jest używana jako środek ściągający, odkażający, przeciwbakteryjny i przeciwpasożytniczy. Celem niniejszej pracy było określenie składu chemicznego i działania antybakteryjnego części nadziemnych *Artemisia aucheri* przeciwko różnym mikroorganizmom, w tym bakteriom Gram-dodatnim i Gram-ujemnym, grzybom nitkowatym i drożdżakom za pomocą metody dyfuzji krążkowej i metodą rozcieńczeń. Działanie antyutleniające olejku eterycznego z *A. aucheri* określono metodą z użyciem DPPH. Stosując GC i GC/MS zidentyfikowano 55 składników olejku i określono ich zawartość w olejku eterycznym *A. aucheri* (stenowiło to 98% całości olejku). Głównymi składnikami były: octan geranylu (17,2%), E-citral (17,1%), linalool (12,7%) i Z-citral (10,5%). Badania działania przeciwbakteryjnego wykazały, że oporny na działanie olejku był *Pseudomonas aeruginosa*, natomiast najbardziej wrażliwe były *Staphylococcus aureus* i *Candidia albicans*. Działanie przeciwutleniające *A. aucheri* jest tak samo silne jak działanie Troloxu. Dla określenia skuteczności działania olejku eterycznego konieczne są dalsze badania.

Słowa kluczowe: *Artemisia aucheri, skład chemiczny, działanie przeciwbakteryjne, działanie przeciwutleniające*